CONDITIONING HYPERPOLARIZATION DELAYS IN SQUID AXON POTASSIUM CHANNELS

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ABSTRACT Activation of potassium conductance in squid axons with membrane depolarization is delayed by conditioning hyperpolarization of the membrane potential. The delayed kinetics superpose with the control kinetics almost, but not quite, exactly following time translation, as demonstrated previously in perfused axons by Clay and Shlesinger (1982). Similar results were obtained in this study from nonperfused axons. The lack of complete superposition argues against the Hodgkin and Huxley (1952) model of potassium conductance. The addition of a single kinetic state to their model, accessible only by membrane hyperpolarization, is sufficient to describe this effect (Young and Moore, 1981).

INTRODUCTION

The first critical test of the Hodgkin and Huxley (1952) model of potassium conductance in nerve membrane was carried out by Cole and Moore (1960), who demonstrated that the delay in the onset of the conductance after a membrane depolarizing voltage clamp step was increased by strong, conditioning hyperpolarization more than could be accounted for by the Hodgkin and Huxley model. Cole and Moore (1960) also suggested that the control and the delayed results superimposed exactly after translation along the time axis. The concept of exact superposition remained unchallenged until Palti et al. (1976) and Begenisich (1979) demonstrated incomplete superposition of potassium currents in the frog node of Ranvier. Moore and Young (1981) reexamined this issue in squid axons, the preparation originally used by Hodgkin and Huxley (1952) and Cole and Moore (1960), with the sucrose gap voltage clamp technique. They reported complete superposition of their records, although they also reported incomplete superposition of potassium currents in crayfish axons with the axial wire voltage clamp technique (Young and Moore, 1981). Shortly thereafter, Clay and Shlesinger (1982) demonstrated incomplete superposition from internally perfused squid axons with the axial wire technique and Schauf (1983) reported similar observations from dialyzed *Myxicola* axons.

The measurements in this report from both perfused and nonperfused squid axons confirm and extend the earlier work of Clay and Shlesinger (1982).

METHODS

Experiments were carried out on perfused and nonperfused squid giant axons (*Loligo pealei*) using standard axial wire voltage clamp techniques with series resistance compensation (Clay and Shlesinger, 1982, 1983). Internal perfusion was implemented by means of a cannula concentric with the axial wire. The temperature in these experiments ranged between 7° and 10°C. It was maintained constant to within ±0.1°C during any single experiment. The external solution used in all experiments contained 390 mM NaCl, 50 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 10 mM Tris-HCl, and 0.5 μM tetrodotoxin (TTX). The internal perfusion experiments employed a perfusate containing 200 mM K glutamate, 50 mM KF, 25 mM K_2HPO_4, and 505 mM sucrose. Glass pipettes of 50–80 μm tip diameter filled with 0.5 M KCl in 1–2% agar were used as internal and external voltage sensing electrodes. Liquid junction potentials were ≤ 3 mV. The potentials used in this report were not corrected for these relatively small voltage offsets.

The holding potential for all experiments was −80 mV. A 50 ms prepulse to −120 mV was used for most of these results so that the kinetics of both the control record and the record following a prepulse to potentials more negative than −120 mV could be clearly separated from the capacitance current transients. Test potentials used were 0, 20, 50, and 100 mV.

RESULTS

The primary observations concerning conditioning hyperpolarization induced delays of potassium conductance kinetics and the lack of superposition of the kinetics after translation of the records along the time axis are illustrated in Figs. 1 and 2. The results in Figs. 1 *A* and 2 *A* were obtained from an internally perfused axon; the results in Figs. 1 *B* and 2 *B* were obtained from a nonperfused, intact axon. The test potential for both experiments was +50 mV. The record labeled *a* in Fig. 1 *A* was obtained with a

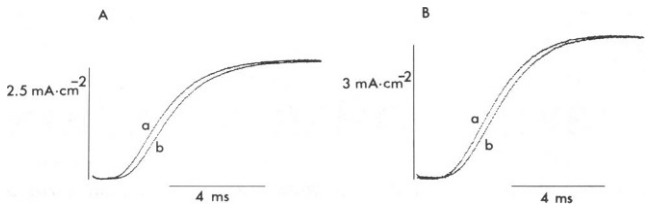

FIGURE 1 Conditioning hyperpolarization induced delays of potassium conductance. (*A*) Record *a* was obtained with a 50-ms duration prepulse to −120 mV followed, by a test depolarization to 50 mV. Record *b* was also obtained with a 50-ms duration prepulse to −120 mV followed by a 5-ms prepulse to −230 mV. Perfused axon C81.67. (*B*) Records *a* and *b* were obtained with a 50-ms prepulse to −120 mV followed by a 5-ms prepulse to −140 mV (*a*) or −230 mV (*b*). Test potential was 50 mV. Nonperfused axon C81.76. Holding potential in both experiments was −80 mV.

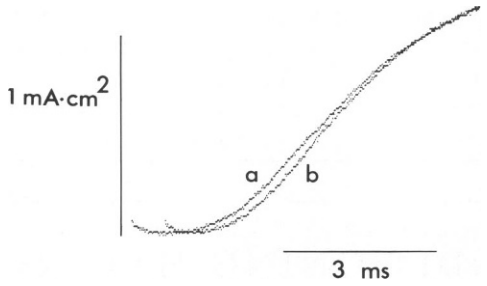

FIGURE 3 Additional results showing lack of complete superposition of potassium current kinetics in a nonperfused axon. Record *a* was obtained with a 10-ms prepulse to −140 mV, followed by test depolarization to 20 mV. No prepulse was used for record *b*. Holding potential was −80 mV. Axon C81.76.

50-ms prepulse to −120 mV. The record labeled *b* was obtained with a 50-ms prepulse to −120 mV, followed by an additional prepulse of 5-ms duration to −230 mV. The results in Fig. 1 *B* were also obtained with a 50-ms prepulse to −120 mV followed by a 5-ms pulse to −140 mV for the record labeled *a*, and a 5-ms pulse to −230 mV for the record labeled *b*. Both experiments clearly demonstrate the delay in the activation of the kinetics produced by conditioning hyperpolarization. The lack of superposition of these results after time translation is illustrated in Figs. 2 *A* and *B*. Fig. 2 *A* illustrates the initial portions of the corresponding records in Fig. 1 *A* on expanded scales with a shift of record *a* along the time axis so as to achieve maximum overlap with record *b*. Fig. 2 *B* illustrates a similar treatment of the records in Fig. 1 *B*. Both experiments show a relatively small, but clear deviation from exact superposition. This effect is more readily apparent for the intact axon in Fig. 3. Record *a* in Fig. 3 was obtained with a 10-ms duration prepulse to −140 mV with a holding potential of −80 mV. No prepulse was used for record *b*. Test potential was 20 mV.

The effect of conditioning depolarizations on activation kinetics is illustrated in Fig. 4. A 10-ms duration prepulse to either −140, −40, −20, or 0 mV was used in this experiment. Test potential was 20 mV. The resulting membrane currents are shown in Fig. 4 *A* without shifts along the time axis. The time shifted results are shown in Fig. 4 *B*. The −20 and 0 mV records superimpose upon the −140 mV record, whereas the −40 mV record does not.

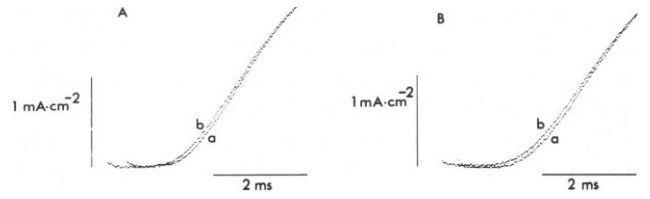

FIGURE 2 Same records as in Fig. 1 on expanded scales, showing lack of complete superposition. Record *a* was translated along the time axis by 0.43 ms in *A* and 0.38 ms in *B*.

DISCUSSION

The results in this report provide further evidence that potassium current kinetics in squid axons with various levels of conditioning hyperpolarization do not exactly superpose, in general, following time translation. The earlier report of incomplete superposition in squid axons by Clay and Shlesinger (1982) was based on results from internally perfused preparations, whereas the original observations of complete superposition by Cole and Moore (1960) and the recent confirmation of this effect by Moore and Young (1981) were obtained from intact axons. Consequently, the difference between the results in Clay and Shlesinger (1982) and the other workers could, perhaps, be attributable to an effect on potassium kinetics of the internal perfusion procedure. The results in this report from both intact and perfused preparations argue against that possibility. Other factors may be responsible for the discrepancy. For example, most of the results published by Cole and Moore (1960) were obtained at $T = 20°C$. Under these conditions the hyperpolarization induced delay is relatively small, which makes it difficult to determine whether or not the kinetics superimpose. Moreover, some of the results from Cole and Moore (1960) at $T = 5°C$ appear not to superimpose.

The lack of superposition reported here can be easily

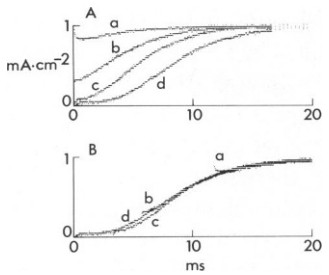

FIGURE 4 Superposition of kinetics with depolarizing prepulses. (*A*) These results were obtained with a 10-ms prepulse to −140 mV (*a*), −40 mV (*b*), −20 mV (*c*), or 0 mV (*d*). Holding potential was −80 mV. Axon C81.70. (*B*) Same results as in *A* with the following time translations: 11.8 ms (*a*); 5.7 ms (*b*); 3.3 ms (*c*).

overlooked. For example, a cursory examination of the records in Fig. 1 might suggest that the control and test kinetics were, in fact, identical, except for time translation. The deviation from exact superposition is apparent only when the records are presented on expanded axes, as in Fig. 2. The results in Fig. 1 of Moore and Young (1981) are not presented with sufficient clarity to argue against this relatively small effect. The results in Fig. 2 of their paper are somewhat more indicative of exact superposition, although a close examination of the initial 2 ms of their results in Fig. 2 *B* suggests a deviation of the test from the control kinetics, which might be more readily apparent on expanded scales. Their results in Fig. 3 were obtained with a test potential of −10 mV. Clay and Shlesinger (1982) also reported complete superposition for a similar test level (0 mV). The deviation from exact superposition is apparent only when test levels more positive than 0 mV are used. In summary, the apparent discrepancy between Moore and Young (1981) and this report may simply be related to a difference in voltage clamp protocol, or the manner in which the results have been presented.

Potassium activation kinetics similar to the results in this report have been observed in frog node of Ranvier (Palti et al., 1976; Begenisich, 1979), crayfish axons (Young and Moore, 1981), and *Myxicola* axons (Schauf, 1983). It is tempting, therefore, to speculate that the lack of exact superposition is a feature common to the delayed rectifier channel in axons from all species. This effect cannot be explained by the original Hodgkin and Huxley model of potassium conductance, although a simple modification of their model, which involves the addition of a single kinetic state accessible only by membrane hyperpolarization, is sufficient to describe the effect (Young and Moore, 1981).

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